IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Application No.:	10/599,660
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Inventor (first named):	SUNWOO, Hoon
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Examiner Name:	WEN, Sharon
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Declaration Under 37 CFR Sec. 1.132

Province of Alberta
CANADA

I, HOON SUNWOO, hereby declare as follows:

1. I received a Ph.D in Food Science and Technology from University of Alberta, Edmonton, Canada in 1998; a M.Sc. in Animal Nutrition and Feeding from Kon-Kuk University, Seoul, Korea in 1992; and a B.Sc. in Animal Husbandry from Kon-Kuk University, Seoul, Korea in 1991. My doctoral thesis related to chemical characterization of growing antlers from Wapiti. I completed postdoctoral work on the antimicrobial effects of immunoglobulin Y at the Alberta Agriculture Research Institute, Edmonton, Canada. I am currently a Research Associate in the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada. My research focuses upon immunoglobulins and their uses for amelioration of celiac disease and osteoporosis. I lecture on immunoglobulin Y production and applications, animal science, and nutraceuticals at the University of Alberta, Edmonton, Canada. I also manage the National Science and Engineering Research Council of Canada for Technology Transfer Partnership Program on antibody farming technology. As shown by my degrees and professional background, I have worked in the fields of animal science, immunology, and natural health products since 1991, and have published more than 50 scientific papers, book chapters, and abstracts.

2. I am a co-inventor of the invention described in the above-identified patent application (the "Invention") and as such have a personal knowledge of the matters hereinafter stated.

3. I make this Declaration in response to rejections of the above noted patent application under 35 U.S.C. §103 citing Lee (U.S. Patent No. 5,367,054) and Ellis *et al.* (Gut, 1998, 43:190-195).

4. Based on my experience and knowledge of immunology and celiac disease, I believe that the pending claims of the present application patentably distinguish the cited prior art by virtue that the claimed IgY polyclonal antibodies have the ability to specifically bind to gliadin, HMG, LMG and mixtures thereof in the gastrointestinal tract of a subject. This ability or activity of IgY polyclonal antibodies specific to components of gluten is not taught or suggested by Lee or Ellis *et al.*

5. To assist the Examiner, I have provided additional evidence to demonstrate that the Invention distinguishes the prior art compositions. I personally performed the experiments described below, and the analysis and conclusions reached were arrived at on the basis of my best professional judgment.

6. Four gluten proteins from wheat, barley, rye and oat were isolated using the method described by Tatham *et al.* (2000). The gluten proteins, which are active in celiac disease and other gluten related conditions, were isolated as prolamins (known as gliadins in wheat, hordeins in barley, secalins in rye and avenins in oat). Two hundred 35-wk-old Single Comb White Leghorn chickens were immunized four times with emulsions of the extracted gluten proteins until hens produced egg yolks with a high titer of specific IgY. A therapeutic composition comprising IgY polyclonal antibodies and a physiologically acceptable excipient was prepared and formed into an oral dosage form (in this embodiment, a capsule). The stability and dissolution of the composition under simulated gut conditions were determined in several tests summarized below.

6.1. Stability test:

IgY contents in capsules with or without excipient (for example, mannitol or sorbitol) maintain a constant amount of IgY up to 50 weeks at room temperature (Table 1):

Table 1. Long term stability of IgY capsule

Content (mg/ml)	Week no:	0%	5%	10%	15%	20%
IgY	1	23.59	22.86	21.43	22.78	22.74
	2	31.37	30.86	35.13	36.57	38.71
	3	22.71	25.96	25.16	27.48	26.24
	4	24.56	26.59	28.93	30.50	31.18
	25	22.51	23.00	26.48	23.68	24.99
	50	24.28	26.26	28.13	28.86	28.58

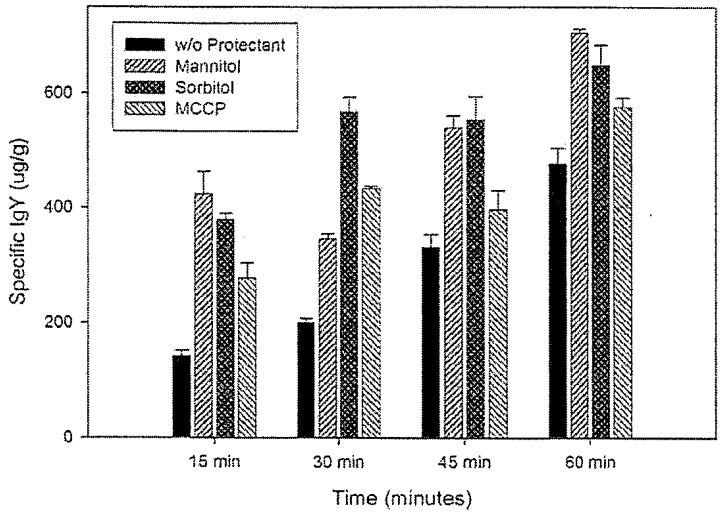
6.2. Disintegration test:

The IgY capsule was studied for disintegration under gastric pH of 1.2. All six capsules were observed to rupture after 2.5 min in the disintegration vessel, whereas a complete disintegration of the six capsules was observed after 30 minutes of exposure. This result suggests that IgY can be released for mixture with the food matrix to bind target gluten.

6.3. Dissolution test:

Compositions comprising IgY polyclonal antibodies and an excipient (20% mannitol, 20% sorbitol or 15% microcrystalline cellulose powder) were used to test the survival of IgY polyclonal antibodies in simulated gut fluid (SGF) and intestinal fluid containing bile salt (SIF). In SGF, the activity of IgY with either mannitol or sorbitol was higher than IgY without excipient and MCCP (Figure 1). IgY without excipient shows the lowest activity of IgY, indicating that excipient protects IgY from enzymatic digestion and low gastric pH. Food normally resides for about one hour in the stomach. After a one hour exposure, the survival rate of IgY was highest at 70.5% and 64.85% in mannitol and sorbitol, respectively. This result suggests that excipient addition may be beneficial and that sufficient IgY remains to enter the small intestine.

Figure 1.



After a one hour incubation of IgY with sorbitol in SIF, IgY was degraded. The activity of IgY was retained at 24.1% of total IgY. Overall, the dissolution results support the survival of IgY polyclonal antibodies in the gastrointestinal tract after ingestion, there by allowing the opportunity for IgY polyclonal antibodies to capture gluten in both the stomach and small intestine.

6.4. In Vitro Assessment of Anti-gliadin IgY Activity

6.4.1. Anti-gliadin IgY activity was evaluated to determine 100% inhibition of gliadin by *in vitro* incubation of IgY capsule and gliadin in gastrointestinal fluid (SGF and SIF). This test estimates the binding ratio of IgY antibody and gliadin under physiological conditions which in turn, determines the dose of IgY antibody to inhibit gliadin in the gastrointestinal tract of gluten-intolerant subjects. A competitive assay of gliadin versus IgY capsule showed that 0.08 g of IgY captured 0.3 g of gliadin in SGF. A competitive assay of gliadin versus IgY capsule showed that 0.08 g of IgY captured 0.015 g of gliadin in the SIF. Two IgY capsules (800 mg IgY formula) may thus neutralize 3 g and 0.15 g of gliadin peptides in simulated stomach and small intestine, respectively.

6.4.2. IgY binding capacity in complex meal conditions was also investigated. Food spiked with gliadin, and IgY capsule were mixed in stomach and small intestine simulated conditions. A

competitive assay of gliadin in 10 g of food matrix versus IgY capsule showed that 0.08 g of IgY captured 0.28 g of gliadin in the food matrix in SGF. A competitive assay of gliadin in the food matrix versus IgY capsule showed that 0.08 g of IgY captured 0.24 g of gliadin in the food matrix. This result suggests that IgY formula with foods was less damaged in the small intestine due to the diluted effect of protein digestive enzymes in SIF.

6.4.3. The above results indicate that two IgY capsules (800 mg IgY composition) can neutralize 3.2 gram of gliadin in foods in simulated stomach and small intestine, respectively. Based on 70% survival of IgY in stomach, two IgY capsules may bind to 2.8 g of gliadin in foods and the remaining IgY may also bind to 2.4 g of gliadin in small intestine, which indicates that two capsules of IgY may bind 5.2 g of gliadin in both stomach and small intestine.

7. In summary, in view of my experiments, I believe that I have demonstrated that the claimed Invention exhibits the activity of being capable of specifically binding to gluten in simulated gastrointestinal tract fluids. This characteristic is not taught by either Lee or Ellis *et al.* Further, the inclusion of an excipient also enhances the survival of IgY polyclonal antibodies in simulated gastrointestinal fluids. Neither Lee nor Ellis *et al.* discloses the addition of an excipient with IgY polyclonal antibodies or the benefits of same.

8. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application or the patent issuing thereon.

September 1st 2009
Date

Hoon Sunwoo